Evaluation Report on the Doctoral Thesis by Anna Tokarenko: „Novel modified nucleosides with antiviral or cytostatic activity.“

The thesis is aimed to the application of the modular methodology to the synthesis of *C*-ribonucleosides and 2´-deoxy-*C*-ribonucleosides bearing substituted benzene, pyridine, piperidine, furan and thiophene rings, which were recently developed in prof. Hocek group for the synthesis of analogous 2´-*C*-methyl-*C*-ribonucleosides. These compounds are structural analogues of Sofosbuvir an inhibitor of HCV RNA polymerase. This methodology is based on the synthesis of key intermediate - *C*-nucleoside containing halogenated aromatic or heteroaromatic group and its further modification by conversion of halogen for other groups by cross-coupling reactions or nucleophilic substitutions.

Even though the synthetic approach was analogous to that previously reported, the chemistry was different. Consequently, the development of the new methodologies and the optimization of most of the reaction steps were necessary. The synthesis started from benzylated 2-*C*-methyl-D-ribono-1,4-lactone. Subsequently, it was reacted with bromolithium reagents giving mixture of anomeric hemiacetals, which were then reduced directly or after conversion to the corresponding acetates to the required *C*-nucleosides bearing 4-bromophenyl, 2-bromopyridin-5-yl and 5-bromopyridin-2-yl groups. This step was quite stereospecific giving only the required β-anomer in most cases. In the next step bromine was converted to the corresponding methyl, amino, dimethylamino and hydroxy derivatives by Pd-catalyzed reactions. Hydroxy derivatives were than methylated to install methoxy group. The final step debenzylation by hydrogenolysis was somewhat tricky due to competing ring-opening reaction. Finally, appropriate conditions were found and all required *C*-nucleosides were prepared. The structure was in several cases confirmed by X-ray structure analysis. Since sugar modified nucleosides may not be efficiently phosphorylated, the corresponding triphosphates were also prepared. However, neither free nor phosphorylated *C*-nucleosides showed any anti-HCV activity.

The second part of the thesis is dealing with the synthesis and biological evaluation of pyrrolo-fused 7-deazapurine ribonucleosides. This was motivated by earlier findings, that the analogous thieno-fused derivatives show promising biological activities. Similarly to the earlier described thieno derivatives the synthesis started from 2,4-dichloropyrimidine, which was zincated at position 3 and subsequently coupled with 2- or 3-iodo-N-methylpyrrole. One chlorine atom was then substituted with azide and the product was thermally cyclized. Thus, the obtained two isomeric pyrrolo-fused 7-deazapurine were then converted to ribonucleosides using Vorbrüggen conditions. Again, the previous reaction conditions for thieno derivatives had to be optimized. The chlorine atom in the obtained benzoyl-protected nucleosides was then exchanged by cross-coupling reactions or direct nucleophilic substitution to furyl, benzofuryl, methyl, dimethylamino, amino, methoxy and methylthio derivatives. The preparation of last four derivatives proceeded with spontaneous debenzylation, the others were debenzylated by treatment with MeONa in MeOH. Due to its high cytotoxicity the 6-methyl derivative was chosen for the study of intramolecular metabolism and mechanism study. The study of the biological activity revealed, that while the pyrrolo derivatives obtained by cyclization of 3-pyrroloderivatives were completely inactive, the isomeric derivatives obtained from 2-pyrroloderivatives bearing methyl, methoxy and methylsulfynyl groups showed submicromolar cytotoxicity. It was find out that the nucleosides are phosphorylated and incorporated to RNA and DNA that leads to double strand breaks and apoptosis. Several compounds also showed submicromolar anti-HCV activity. The results indicate that the pyrrolo-fused 7-deazapurine nucleosides are good candidates for further studies.

From the formal point of view, the thesis is divided as it is usual in this type of works. The introduction describes basic applications of deazapurine derivatives and *C*-nucleosides in medicinal

chemistry, and the synthetic approaches to *C*-nucleosides. The part “Experimental section” describes experimental details by the way which is usual in publications. All compounds are fully characterized. The References part contains more than 250 references. The thesis is written clearly, very concise and practically without formal mistakes. The few I have found are mentioned below.

It is not practical, when the compounds in the part “Introduction” are referred by names and not by numbers as in the second part. It is then difficult to find the corresponding structures when it are mentioned again later in the text.

Pg. 16: fabacavir

Pg. 19: penciclovir is not carbocyclic analogue

Pg. 66: Instead of “Furan-2-yl and benzofuran-2-yl should be furan-3-yl … .

I have the following questions:

Do you have explanation why only one of two possible isomers is formed by cyclization of 3-pyrrolyl derivative **37**?

Has the lower selectivity in the reduction of anomeric mixture of 5-bromopyridin-2-yl derivatives **13** and **14** compared to 2-bromopyridin-5-yl derivatives **10** been observed also previously in the ribonucleoside series? Is there any explanation for this?

Overall, the thesis of Anna Tokarenko fulfils the criteria for the PhD thesis. She proved that she is able of an independent scientific work, which is demonstrated by two publications in high rated journals of the field. Therefore, I recommend her thesis for the defence and further proceedings for obtaining the PhD degree.

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